November 12, 1953

Dr. R. G. E. Murray
Department of Bacteriology
University of Western Ontario
Victoria Hospital
London, Ontario, Canada

My dear Murray:

Thank you very much for your courtesy in sending the reprints and draft just received.

I have not yet had time to study your remarks more carefully, but am excited to see how well they have anticipated the conclusions that I am just now beginning to crystalize from studies with E. coli K 12. I was impressed to notice in our material that after Giemsa staining every so often unhydrolysed cells would show a red nuclear staining against a very dark blue-purple cytoplasm. The nuclei, unfortunately, not too sharp, appeared here generally sac-like and not like the discrete granule after hydrolysis. Perhaps most of the nuclei are vascular consisting of a hypo-chromatic "matrix" in which the Feulgen-positive acid-resistant granules are embedded. I am, however, inclined to regard these granules as possibly hyper-chromatic blocks in the chromosome spiling. I could not readily reconcile most of the granule figures with a mitotic sequence, but if the figures are comparable to the chromocenters of interphase nuclei of higher forms the contradictions may still be resolvable. On the other hand, occasional figures are compelling reminiscent of mitotic separation. However, I do not see spindles. If there is something comparable to mitosis (and of course, there has to be if only to comprehend haploid and diploid K 12) perhaps the spindle or its mechanical analog is confined within the nuclear membrane as it is in so many Protista. (One of the chief reasons I had been suspicious of attempts to force all of the granule figures into a mitotic mold is that I could often see connections between the separate "chromosomes.") In a few fortunate cases these seem to have been strung out as in the enclosed prints. (By the way, I had thought that the Giemsa technique called for diluting the strain in rather dilute buffer at pH 7. I had a lot of trouble on this count when I sought to use strong enough buffer to actually control the pH of the stain solution. This should be about 5 not 7. I now use the commercial stain diluted in M/10 pH 5 phosphate buffer 1:10 for 10 to 15 minutes. Abopon diluted 2:1 in buffer and filtered at 60 degrees has proved to be an indispensable mounting agent (following Delaporte), with occasional trouble from crystallization.

We still are primarily concerned with the mating problem rather than these questions of nuclear structure though I find it difficult to see how we will be able to avoid the latter. Though we have little new direct cytological evidence on mating, we now have fairly direct demonstrations that the synkaryons have the full genetic complement of both parents although there are later eliminations that influence segregation ratios.

Yours very sincerely,

JL/gw

Joshua Lederberg